STUDIES ON THE PULLORUM DISEASE II. ON THE FLUCTUATION OF THE AGGLUTININ TITRE OF THE FOWL

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By

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With regard to the rise and fall of the agglutinin titre of the fowl infected with *Sal. pullorum*, N. Imai et al. (1)(2), in several papers, have discussed the significance of the phenomenon. Iwamori et al. (3) observed on the attitudes of the fowls to the rapid agglutination. Edwords and Hull(4), in their monthly tests during a year on a group of reactors, observed only slight variations in the agglutination. In our previous paper (5), it was reported that in winter new positive reactors of the rapid whole blood agglutination appeared rarely and fowls, which had showed the positive reaction, also tended to become the negative reactor. It has been suggested that those facts are important in the control of the pullorum disease with the rapid blood agglutination.

In this paper, we attempted further observation on the problem and to detect the beginning of the appearance of the agglutinin in serum at the early stage of the natural infection. The ability of the production of the agglutinin at the bady age was also studied experimentally.

Materials and methods

Thirty-two New Hampshire chicks which recovered from the pullorum disease at baby age and acquired resistance to acute infection were used for observations on the fluctuation of the agglutinin titre. This infection broke out on May 10th, 1953 (3 days old) and 37 per cent of the 107 chicks died from the disease within a month. All chicks employed had *Sal. pullorum* in their droppings at the severe stage of the infection. The tests were carried out weekly as a rule from May 24th, 1953 (17 days old) to July 16th, 1954 (434 days old).

The agglutination was carried out by means of the rapid serum test, instead of the rapid whole blood test which was used in our previous experiments, with the commercial stained antigen and by the quantitative tube method with antigens made in our laboratory. The rapid test was performed

Table 1. Six types in the attitudes of the fowls to the repeated agglutination in the natural infection.

Type	Chicks	Date of inspection													Autopsy		
		1953								1954					Date	Reaction	*Sal. pullorum* recovered
		V*	VI	VII	VIII	IX	X	XI	XII	I	II	III	IV	V			
		24	1 8 15 22 29	6 13 20 27	3 10 17 24 31	7 14 28	5 12 20 28	4 10 16 26	14 24	6 15	1 12 19 26	5 12 19 26	2 15 23 29	7 14 21 28			
I	No. 156 ♀	−	+ + + + +	+ + + +	+ + + + +	+ + +	+ + + +	+ + + +	+ +	+ +	+ + + +	+ + + +	+ + + +	+ + + +	28. V./54	+	Yes
	No. 136 ♀	−	+ − − + −	± + + +	+ + + + +	+ + +	+ + + +	+ + + +	± +	+ +	+ + + +	+ + + +	+ + + +	+ + + +	2. VII./54	+	Yes
	No. 126 ♂	−	+ + + + +	+ + + +	− + + + +	+ + +	+ + + +	+ + + +	+ +	+ +	+ + + +	+ + + +	+ + + +	+ + + +	4. VI./54	+	Yes
	No. 141 ♀	−	− + + − +	+ + + +	− + + + +	+ + +	+ + + +	+ + +							16. XI./53	+	Yes
	No. 149 ♀	−	− + − + −	+ + + −	+ + + + +	+ + +	+ + + +	+ + + +	+ +	+ +	+ + + +	+ + + +	− − − +	+ + + +	16. VII./54	+	Yes
	No. 125 ♀	−	± + + + −	− − − +	+ + + + +	+ + +	+ + + +	± − ±	− +	+ +	+ + + +	+ + + +	+ + + +	+ + + +	1. VIII./54	+	Yes
II	No. 129 ♀	−	− − − + −	+ + + −	+ + + + −	− + +	+ − − +	+ + − −	− +	+ −	+ − − −	+ + + −	− + + +	+ − − −	6. VI./54	+	Yes
	No. 142 ♀	−	+ + + + +	+ + − −	− − + + +	+ + −	− − + −	+ + ± ±	− −	+ +	− − − +	+ + + +	+ − − −	+ + + +	4. VI./54	+	Yes
	No. 145 ♂	−	+ − + + −	− − + +	+ − − + +	− − ±	+ + + +	− −							10. XI./53	−	Yes
	No. 147 ♀	−	− + + + +	+ − − +	− − − + +	+ + −	− − − ±	− − − −	− −	− −	− − + +	+ + − −	− + − +		29. IV./54	+	Yes
III	No. 133 ♀	−	− − − + −	+ + + −	+ + + + +	− − −	− − − −	− − − −	− −	− −	− − − +	− − − −	− − − −	− − − −	16. VII./54	−	Yes
	No. 137 ♀	−	± + − + +	− − − −	− − − − −	− − −	− − − −	− − − −	− −	− −	− − + +	+ + − −	− − − +		29. IV./54	+	Yes
	No. 134 ♀	−	− − ± − −	− − − −	− − − − −	− − −	− − − −	− − − −	− −	− −	− − − −	+ − − −	− + − +	− − − −	20. VII./54	+	Yes
	No. 143 ♀	−	− − ± − −	− − − −	− − − − −	− − −	− − + −	− − − −	− −	− +	− − − −	− − − −	− − − +	− − + +	6. VI./54	+	Yes
	No. 154 ♀	−	+ − − − −	− − − −	− − − − −	− − −	− − − −	− − − −	± ±	+ +	+ + + +	+ + + +	+ + + +	+ + + +	14. V./54	+	Yes
IV	No. 127 ♂	−	− + − + +	− − − −	− − − − −	− − −	− − − −	− − − −	− −	− −	− − − −	− − − −	− − − −	− − − −	5. VI./54	−	No
	No. 128 ♂	−	+ + + ± −	− − − −	− − − − −										31. VIII./54	−	No
	No. 132 ♂	−	+ − − + +	− − − −	− − − − −	− − −	− − − −	− − −							16. XI./53	−	No
	No. 140 ♂	−	− + + + +	− − − −	− − − − −	− − −	− − − −	− − − −	− −	− −	− − − −	− − − −	− − − −	− − − −	9. VII./54	−	No
	No. 146 ♂	−	+ − − − −	− − − −	− − − − −	− − −	− − − −	− − − −	− −	− −	− − − −	− − − −	− − − −	− − − −	6. VI./54	−	No
	No. 135 ♂	−	− − − − +	± − − −	− − − − −	− − −									28. IX./53	−	Yes
	No. 139 ♂	−	− − − + −	− − − −	± − − − −	− − −									28. IX./53	−	Yes
	No. 144 ♀	−	± − − ± −	− − − −	− − − − −	− − −	− − − −	− − − −	− −	− −	− − − −	− − −			19. III./54	−	No
	No. 151 ♂	−	± − ± − −	− − − −	− − − − −	− − −	− − − −	− − − −	− −	− −	− − − −	− − − −	− − − −	− − − −	28. V./54	−	No
	No. 130 ♀	−	± − ± − −	− − − −	− − − − −	− − −	− − − −	− − − −	− −	− −	− − − −	− − − −	− − − −	− − − −	28. V./54	−	No
V	No. 155 ♂	−	− − − − −	− − − −	− − − − −	− − −	− ± − −	− − − −	− −	− −	− − − −	− − − −	− − − −		29. IV./54	−	No
	No. 152 ♀	−	− − − − −	− − − −	− − − − −	− − −	− − − −	− − ± −	− −	− −	− + − −	− − −			19. III./54	−	No
	No. 153 ♂	−	− − − − −	− − − −	− − − − −	− − −	− − − −	− ± − −	− −	− −	− − − −	− − − +	− − − −	− − − +	2. VIII./54	+	Yes
VI	No. 150 ♂	−	− − − − −	− − − −	− − − − −	− − −	− − − −	− − − −	− −	− −	− − − −	− − − −	− − − −	− − − −	6. VI./54	−	No
	No. 138 ♂	−	− − − − −	− − − −	− − − − −	− − −	− − − −	− − − −	− −	− −	− − − −	− − − −	− − − −	− − − −	2. VII./54	−	No
	No. 131 ♂	−	− − − − −	− − − −	− − − − −	− − −	− − − −	− − −							16. XI./53	−	No
	No. 148 ♂	−	− − − − −	− − − −	− − − − −	− − −	− − − −	− − − −	− −	− −	− − − −	− − − −	− − − −		29. IV./54	−	No

* Ages of the chicks were 17 days old.

at room temperature, 19° to 28°C, and reading of the reaction was done as in the previous report (5). In the quantitative method, test tubes containing serum and antigen were placed in an incubator for five fours then kept at 20°C for 13 hours. After the reaction occurred sufficiently, the reading was conducted with a magnifying-glass. Several strains were used for the antigens in the tube method. The recently isolated strain from the chicks was added to an basal antigen consisting of two strains M-28 and TK1.

The isolation of the causative bacteria was performed from the blood, liver, spleen, kidney, lung, ovary, testis and intestinal contents. Each material was homoginated in bouillon[1] and bovine bile to be incubated for 24 hours. After then the isolation was carried out with SS agar. The whole of each organ except for the blood and intestinal contents was employed for the incubation.

Experiment on the production of the agglutinin at the baby age was carried out with a flock of 23 baby White Leghorn. Strain M-28 was used as killed or living vaccine. Moreover, an effect of adrenaline on the agglutinin level in serum of the naturally infected fowl was observed.

Results

Thirty-two chicks which recovered from the severe infection of *Sal. pullorum* at the baby age showed six types in attitudes to the repeated rapid serum tests during about one year as given in Table 1. These are as follows (see also Table 2).

Type I ..... They began to react positively when the time since they had recovered from the disease was comparatively short and continued to keep the reaction for a long period as no. 156, or showed negative reaction rarely as nos. 136 and 149. Six fowls belonged to this type, that is 19 per cent of the total.

Type II ..... They began to react positively at the early stage like Type I, but thereafter showed negative and positive reactions alternately of which the durations were almost equal. Four fowls belonged to this type, that is 12 per cent.

Type III ..... They began to react positively or doubtfully at the early stage and then continued negative reaction for a comparatively long period. Afterwards they reacted positively again. Five fowls belonged to this type, that is 16 per cent.

Type IV ..... They reacted only at the early stage, positively or doubtfully, and then continued negative reaction. Ten fowls belonged to this type, that is 31 per cent.

1) Consisting of pepton 1 per cent, meat extract 0.5 per cent, yeast extract powder 0.3 per cent and NaCl 0.5 per cent.

Type V ..... They did not react at the early stage and after continuation of the negative for some time they reacted positively or doubtfully. Three fowls belonged to this type, that is 9 per cent.

Type VI ..... They showed no reaction. Four fowls belonged to this type, that is 12 per cent.

The results of the isolation of *Sal. pullorum* from these types are summarized in Table 2. From all of them belonging to Types I, II and III, the causative bacteria were isolated but not from Type VI at all. The fact that the bacteria were isolated from 20 and 33 per cent of Types IV and V respectively must be given attention in the control of the pullorum disease. If two fowls (nos. 135 and 139) in Type IV, from which the disease germ was recovered, had not been killed and had lived for a long time, they would have become Type III.

Table 2. Number of the fowls belonging to each of the types and results of the isolation of *Sal. pullorum.*

Type	Number of fowls			
	belonging to each type		*Sal. pullorum* recovered	
I	♂ 1, ♀ 5	6 (19%)	♂ 1, ♀ 5	6 (100%)
II	♂ 1, ♀ 3	4 (12)	♂ 1, ♀ 3	4 (100)
III	♂ 0, ♀ 5	5 (16)	♂ 0, ♀ 5	5 (100)
IV	♂ 8, ♀ 2	10 (31)	♂ 2, ♀ 0	2 (20)
V	♂ 2, ♀ 1	3 (9)	♂ 1, ♀ 0	1 (33)
VI	♂ 4, ♀ 0	4 (12)	♂ 0, ♀ 0	0 (0)
Total	♂ 16, ♀ 16	32 (100)	♂ 5 (31%), ♀ 13 (81%)	18 (56)

The first three types consisted of 12 females and three males, and the other three types consisted of one female and 13 males, that is, the former involved 75 per cent of the females and the latter 81 per cent of the males. The bacteria were isolated from 31 per cent of the males and from 81 per cent of the females.

Table 3 gives the percentage of the new positive reactor at each inspection. No chick showed the positive reaction until June 1st, when the chicks were 25 days of age. At this time, the negative reactors were already 56 per cent of the total number of fowls. After then the positive reactors in-

creased and at 53 days old the negative reactors which had shown no reaction attained 22 per cent of the total. Thirty-eight and 29 per cent of the total positive reactors began to show the reaction at 25 and 32 days old respectively, and 84 per cent of them appeared before 53 days old. Of both reactors, doubtful and positive, 90 per cent of them came out by that time. Four fowls which are given by the difference between total of new positive reactors and that of new positive and new doubtful reactors did not change to the positive through out this experiment.

Table 3. Number of the fowls which began to show the positive reaction at each inspection with the rapid serum test.

Date of inspection	1953										1954			Total reactors
	May	June					Oct.		Nov.		Feb.	March		
	24	1	8	15	22	29	12	20	10	16	12	5	26	
Age in days	17	25	32	39	46	53	158	166	187	193	281	302	323	
New positive reactor[1]	0	9 (38)	7 (29)	0	3 (13)	1 (4)	0	1 (4)	0	0	1 (4)	1 (4)	1 (4)	24 (100)
New positive and new doubtful reactors[2]	0	14 (50)	5 (18)	2 (7)	3 (11)	1 (4)	1 (4)	0	1 (4)	1 (4)	0	0	0	28 (100)
Negative reactor[3]	32 (100)	18 (56)	13 (41)	11 (34)	8 (25)	7 (22)	6 (21)	6 (21)	5 (17)	4 (14)	3 (13)	3 (12)	3 (13)	
Total fowls	32	32	32	32	32	32	29[4]	29	28[5]	28	25[6]	25	23[7]	

Number in ( ) indicates percentage. New reactors did not appear at other inspections than those presented in this table.

1) It involves also those that had reacted doubtfully once.
2) New doubtful reactor do not involve those that had reacted positively once.
3) It means those which continued to react only negatively and the percentage is that against the total fowl.
4) Three fowls which had shown positive reaction once were killed before this inspection.
5) One fowl which had shown positive reaction was killed before this inspection.
6) Two fowls which had shown positive reaction and one fowl which had been continually reacting negatively were killed before this inspection.
7) Two fowls which had been a positive reactor and a doubtful reactor were killed before this inspection.

Percentages of the positive, doubtful and negative reactors against the total at each inspection are shown in Fig. 1. Because of the fact that nine out of 32 fowls were killed to be tried the isolation of *Sal. pullorum* at various stages of the infection, these were calculated with the remaining 23 fowls as total. The percentages of the positive were between 20 and 40 at 33 out of 41 inspections since the positive began to appear. They varied within rejection limits, 29.8 ± 8.2 per cent (significance at 5 per cent level), or 29.8 ± 11.0 per cent (significance at 1 per cent level). So three peaks (on June 22nd, March

5th and April 29th) and one bottom (from November 16th to December 14th) were significant at 1 per cent level and the other two peaks (on June 8th and August 24th) were also significant at 5 per cent level. The negative varied

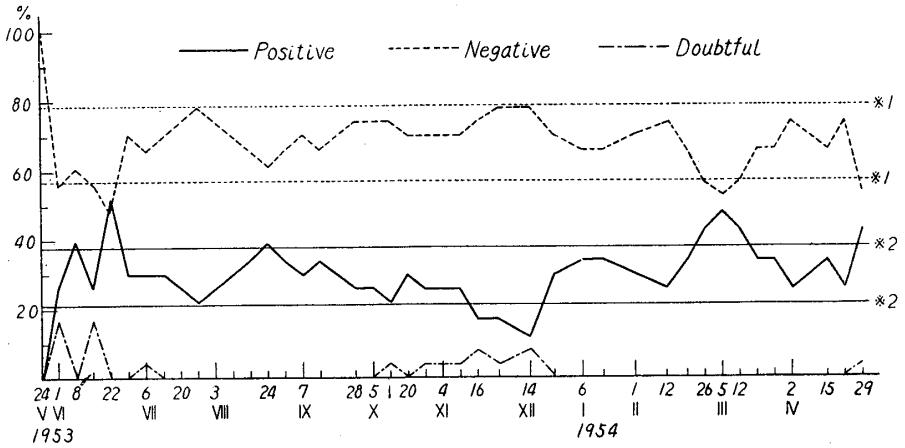

Fig. 1. Percentage of the reactor at each inspection, calculated from 23 fowls existing for long periods.
※ 1. Rejection limits of the positive, 29.8±8.2 per cent (5 per cent level).
※ 2. Rejection limits of the negative, 67.9±10.8 per cent (5 per cent level).

between 67.9 ± 10.8 per cent (5 per cent level) during this experiment and four bottoms (on June 1st, from 15th to 26th of July, from February 26th to March 12th and April 29th) were significant at 5 per cent level. The doubtful reactor appeard intermittently at the early, middle and end of this experiment.

The change of the reaction of the fowls which reacted positively on June 22nd (46 days old) when the positive were most abundant in this experiment is shown in Fig. 2. Curves indicate the percentages of the three kinds of the

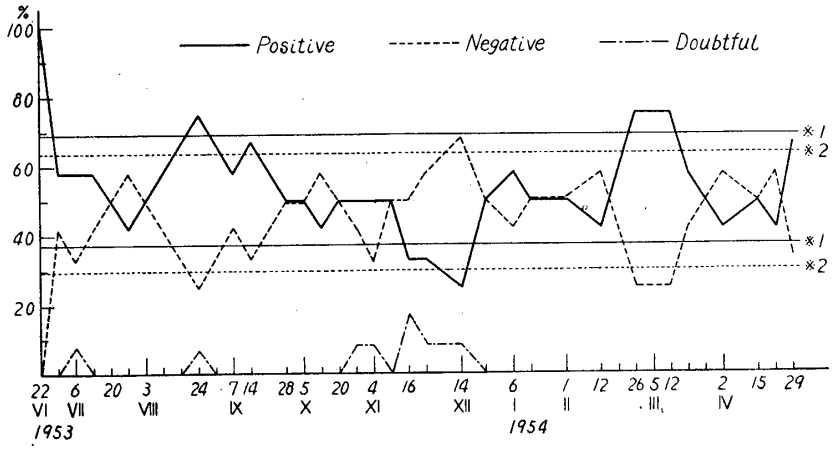

Fig. 2. Change of the reaction of the fowls which reacted positicely on June 22nd, 1953 (46 days old). Percentages of the reactor at each inspection are indicated.
※ 1. Rejection limits of the positive, 53.1±19.5 per cent (5 per cent level).
※ 2 Rejection limits of the negative, 46.6±16.8 per cent (5 per cent level).

reactors against the total at each of the inspections. These were calculated with 12 fowls which had lived through the experiment. The positive began to appear on June 1st and increased rapidly. Immediately after they reached 100 per cent, they tended to decrease and varied within 53.1 ± 19.5 per cent (5 per cent level significance) in most cases, except for three cases, on August 24th, from November 16th to December 14th and from February 26th to March 12th. That is, the first and the third of the exceptions indicate values higher than the limits and the other indicates one lower than the limits. While, after all of the fowls showed the positive reaction, the percentages of the negative varied between 46.6 ± 16.8 per cent (5 per cent level significance) and gave symmetrically two bottoms and a peak against the peaks and bottom in the case of the positive, respectively. The doubtful reactor appeared rarely at low rates, but had an effect on the decrease of the positive in the period from November 16th to December 14th.

The results of the quantitative tube test are as follows. The tests were caried out from August 24th, 1953 (79 days old) to July 16th, 1954 (434 days old) at a week interval as a rule, when the rapid serum tests were also done at the same time. Fluctuation of the agglutinin titre in the serum of each fowl is given in Fig. 3. Though the titres usually rose and fell within short periods and hardly continued to keep constant levels, this attitudes are almost peculiar to the types classified by the rapid serum test. That is, fowls of Type I usually continued to keep high titre, Type II showed comparatively high titre but was often negative, Type III was negative or sometimes showed low titre in the early stage and then gradually became to often exhibit low or high titre, and Type IV showed low titre in the early stage only and then became negative. Types V and VI continued to be negative showing rarely low titre except for no. 153 which afterwards showed high titre. In the two types, I and II, lower titre occurred frequently during from November to January or to February except for a few fowls as no. 156. In the other types the titres appeared very rarely in the same period.

In the average titre, as shown in Fig. 4, Type I gave usually titre higher than 1 : 100 except for three inspections in November and December. Type II in most cases gave titre between 1 : 20 and 1 : 100 with several exceptions in which the titre decreased under the level at six inspection in October, November and February and rose over the level at two inspections in September and October. The average titre of Type III did not increase more than 1 : 20 until January, after which it gradually increased to a high level. In the other three types, titres in average were usually under 1 : 20 except some cases in which they rose up over the level in February and after May in Type V and in April in Type VI. These types showed no titre in December and January.

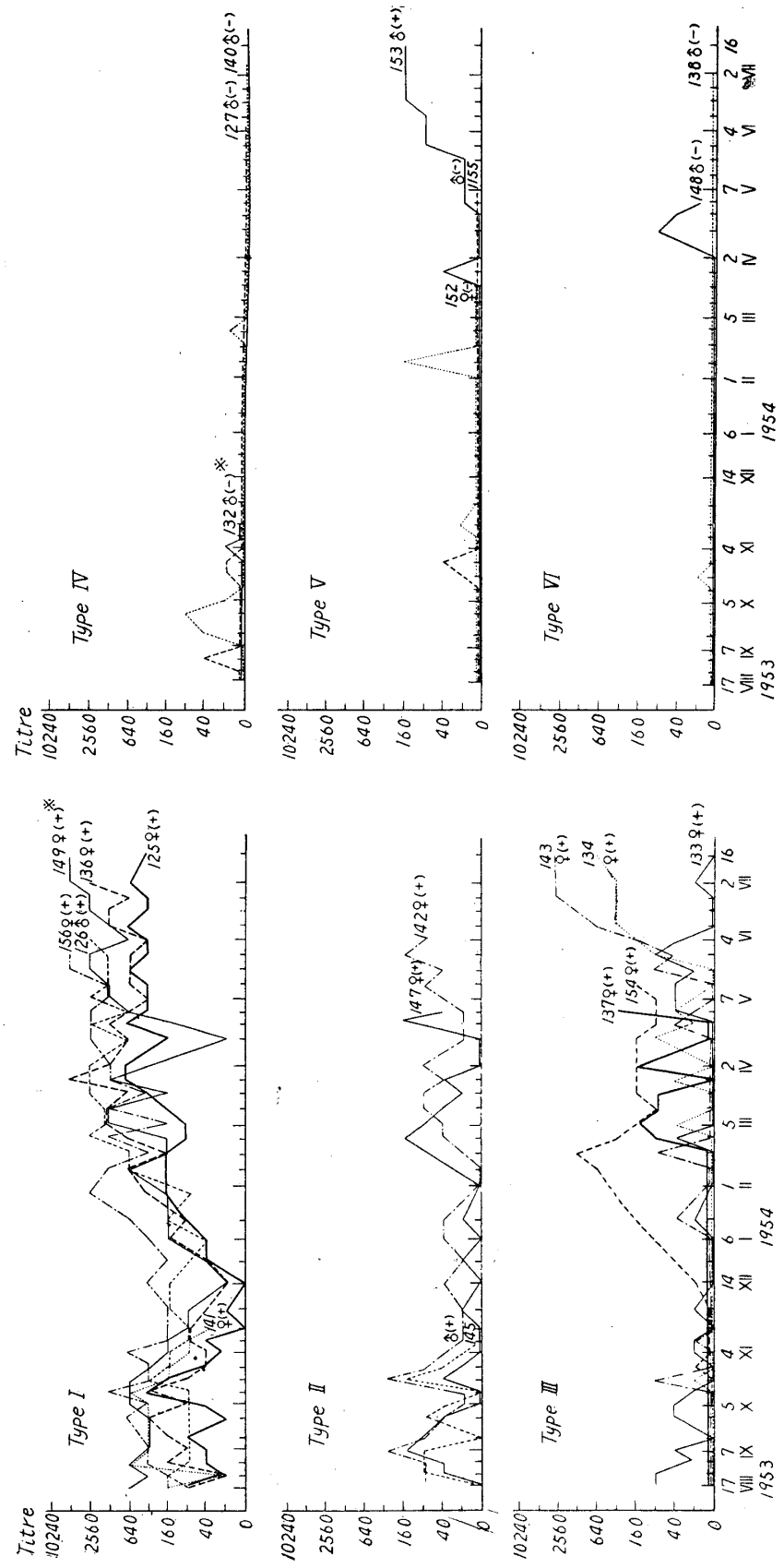

Fig. 3. Fluctuation of the agglutinin titre in serum of the naturally infected fowls.
※ Marks of (+) or (−) indicate results of the isolation of *Sal. pullorum*.

Correlation of the results of the tube and rapid methods obtained from a total of 520 tests for 40 inspections which were conducted simulutaneously

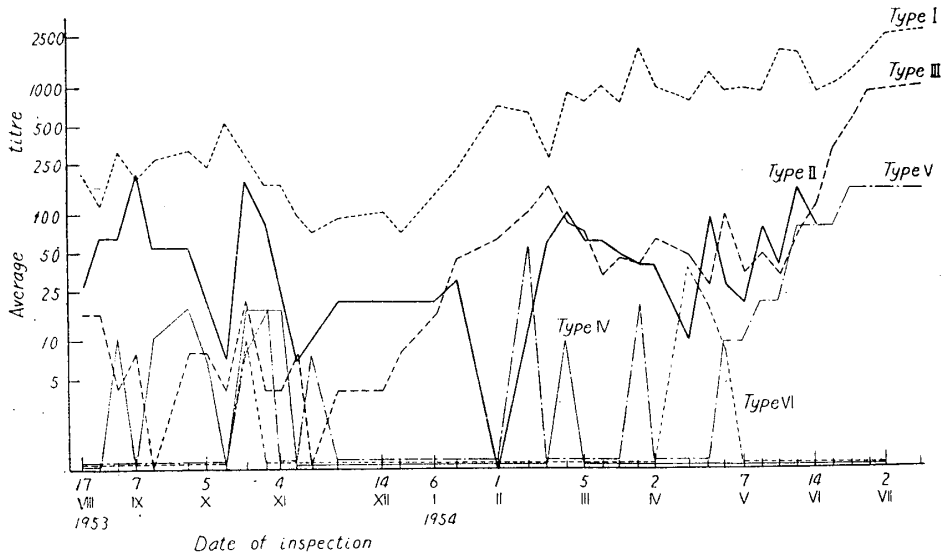

Fig. 4. Average titre of the agglutinin in each type.

with both methods, are shown Table 4. All fowls which reacted positively by the rapid method showed also agglutinin titre by the tube method and 72, 18 and 10 per cent of the doubtful reactors of the rapid method were 1 : 20 to 1 : 40, 1 : 80 and none in titre, respectively. Eighteen-two per cent of the negative reactors of the same method showed no titre by the tube method, and 10 and 8 per cents of them were respectively 1 : 20 and 1 : 40 to 1 : 160 in titre. That is, most of the positive reactors in the rapid test showed titre higher than 1 : 40, most of the negative were lower than 1 : 20 and the titres of many of the doubtful were 1 : 20 and 1 : 40. Though these facts indicate that the results from both methods agreed very closely but not completely, it must be noted that there were negative reactors in the fowls showing comparatively high titres, 1 : 40 to 1 : 160.

Table 4. Relationship between the titre by the quantitative tube method and the reaction by the rapid serum method.

Titre by quantitative tube method	Reaction by rapid serum method			Sum total
	+	±	−	
320	49%	0%	0%	
160	19	0	1	
80	18	18	3	
40	10	36	4	
20	4	36	10	
0*	0	10	82	
Total tests	167	11	342	520

* Negative reaction at the rate of dilution 1 : 20.

However, the results of both tests at autopsy mostly agreed with that of the isolation of *Sal. pullorum* as given in Table 5.

Table 5. Relationship between the agglutinin titre and the results of the isolation of *Sal. pullorum* at the autopsy.

Rapid method		Tube method		Recovery of *Sal. pullorum*	
Reaction	No. of fowls	Titre	No. of fowls	No. of fowls	Total
+	14	320	9	9	14 (100%)
		160	2	2	
		80	1	1	
		40	2	2	
−	18	20 ※	1	0	4 (22%)
		0	17	4	

※ No reaction at the rate of the dilution, 1:20.

All positive reactors by the rapid test from which *Sal. pullorum* was isolated were higher than 1:40 in titre, while all of the negative, of which the disease germ was isolated from 22 per cent, were lower than 1:20, most of them had no titre. It must be considered in the prevention of this disease that there were the none reacting fowls from which *Sal. pullorum* were isolated.

Though K. Suzuki (6) demonstrated that adrenalin injection affected temporarily increase of the agglutinin level and suggested that this treatment was beneficial in the discovery of the none or weakly reacting carrier, in our experiment with small specimens such an effect was not seen as shown in Table 6. Five chicks, 74 days old, of the same flock as in the above experiment were employed. Their agglutinin levels were measured three times every 30 minutes after being injected intramuscularly with 0.1 cc of 0.1 per cent of epinephrine hydrochloride. One chick (no. 141) increased in the titre and two chicks (nos. 133 and 149) decreased in the titre for 30 minutes after injection, and the other two chicks (nos. 132 and 136) did not change their titres. That is, adrenalin may not always increase the agglutinin level.

Table 6. Effect of adrenalin injection of the agglutinin titre in serum of the chicks.

Chicks	Body wt.	Treatment of adrenalin intramuscularly			
		Before injection	After injection		
		10 min.	30 min.	60 min.	90 min.
No. 132	650	0 ※	0	0	0
No. 133	750	200	100	200	200
No. 136	800	400	400	400	400
No. 141	500	400	800	400	400
No. 149	750	800	400	400	800

※ Negative reaction at the rate of dilution 1:25.

As it was mentioned above that the fowls in the natural infection began to react positively from 25 days of age, chicks before this age may not have the ability to form agglutinin so much as can be detected. The following experiment was made with regard to this problem.

Twenty-three chicks obtained from a pullorum-free flock were used. They were divided into four groups as shown in Table 7. Vaccinations were performed three times at every four days with 0.2 cc of killed vaccine[1] of *Sal. pullorum* (M-28) subcutaneously excpt that one chick in each of the treated groups was injected with 0.2 cc of living cells instead of the killed one at the last vaccination. This commenced from 2 days old in the first group, 10 days old in the second group and 18 days old in the third group. The other group was untreated and used as a control. Two mature rats as another control were treated with killed vaccine in the same manner. After vaccination the finished agglutinin titre was measured by the tube method every four days as a rule.

Table 7. Ability of agglutinin formation of the chick by vaccinations of *Sal. pullorum*

Group	No. of chick	Treatment and agglutinin titre — Age in days									Maximum titre shown by individual animals		
		2	6	10	14	18	22	26	30	34	0[2]	10–20	>40
1	5	KV[1]	KV	KV	0[2]	—	10–20 (3)[3]	20 (3)	10–20 (2)	0	2	3	0
	1	KV	KV	LV[1]	0	—	40	160	320	80	0	0	1
2	5			KV	KV	KV	20 (4)	20–40 (4)	10–40 (3)	10 (1)	1	3	1
	1			KV	KV	LV	0	20	160	320	0	0	1
3	5					KV	KV	KV	20–40 (3)	40–80 (2)	1	2	2
	1					KV	KV	LV	80	160	0	0	1
4	5				10 (2)	—	20 (1)	10–20 (2)	10–20 (2)	20 (1)	2	3	0
Mature rat	2	7 days after treatments of killed vaccine									0	0	2[4]

1) KV and LV mean killed and living vaccine in treatment respectively.
2) No reaction at the rate of the dilution 1 : 10.
3) Numbers in ( ) indicate the number of the chicks in which agglutinin titre was detected.
4) Both rats showed titre, 1 : 320.

1) By heating at 80 C. for 30 minutes.

As shown in Table 7, agglutinin levels of the chicks treated with only killed vaccine before 10 days old (group 1) were lower than 1 : 20, similar to those of the untreated control. It is perhaps not of specific reaction. One of the chicks treated with the same vaccine after 10 days old (group 2) and two after 18 days old (group 3) rose over 1 : 40 in titre. The administration of the living vaccine in every group made the titre rise to 1 : 160 but the maximum titre did not appear until 30 or 34 days old. The first appearance of the specific reaction (more than 1 : 40) was at 22 days old. Two mature rats treated in the same way as the chicks increased the titre till 1 : 320 for a week after the vaccination. From all the chicks treated with the living vaccine the treated cells were recovered and in the other case *Sal. pullorum* was not recovered.

Discussion

On the ability of the agglutinin formation in the baby age.

In the case of the natural infection which broke out from 3 days old the positive reactor to the rapid test did not appear until 25 days old (Table 1). Before the test began at 17 days old, five chinical and five subclinical chicks, from which *Sal. pullorum* were isolated, of the same flock were tested at 10 days of age and they showed the negative reaction. In the artificial case, the killed vaccine administered to baby chicks before 10 days old resulted in no detectable formation of specific agglutinin and the appearance of the agglutinin could not be recognized before 22 days old in any vaccination, even the living vaccine (Table 7). Y. Mizuma (7) demonstrated that chicks two months old increased agglutinin as high as 1 : 160 four days after the first intravenous injection and to 1 : 2560 four days after the second injection which were performed every five days with the same vaccine as we used. These facts indicate that the baby chicks of such an age have less ability to form the antibody. Their tissues may make no response to the stimuli of the antigens, or the antibody may be not derived out of the tissuse into the serum if the tissues produce a little antibody. This suggests one of the reasons why the severe infection occurs frequently in the baby chicks.

On the attitudes of the naturally infected fowls to the agglutination

No chick showed positive reaction until 25 days old. Afterwards chicks which began to react positively increased and 84 per cent of the positive reactor appeared till 53 days old, while the negative reactors which had shown no reaction reached 22 per cent of the total fowls by this time. Thirty-eight per cent and 29 per cent of the total positive reactors began to show the reaction at 25 and 32 days old, respectively. These values were higher than those obtained from the inspections carried out at other ages (Table 3). This result is slightly different from that of H. Iwamori (3) who stated that most of the

new positive reactors appeared on 40 and 50 days of age. However it is evident that most of them are apt to appear during from one to two months old.

The percentage of the positive reactors against the total fowls at each inspection reached the maximum (53 per cent) at 46 days of age and then decreased till a level having a fluctuation of 29.8 ± 11.0 per cent. Sometimes they ran away from the limits so that the significant increase appeared at the ages of 79 days (in August), 302 days (in March) and 357 days (in April), and the significant decrease appeared from 193 to 221 days old (November to December). On the other hand, the negative varied between 67.9 ± 10.8 per cent showing four bottoms of the curves which appeared to be contrary to the peaks of the positive (figure 1).

The fowls which reacted positively at 46 days old when the positive were most abundant in this experiment became gradually negative reactor. Though the positive varied between 53.1 ± 19.5 per cent in most cases, they ran away from the limits in three cases which appeared at 109 days old (in August), from 193 to 221 days old (November to December) and from 295 to 309 days old (February to March). The first and the third cases were significant increases and the other was a significant decrease.

As was above mentioned, it is certain that a number of the pullorum-carriers in a flock which can be detected by the rapid test always fluctuates without keeping a constant level. This phenomenon will become more evident by comparing with the data obtained from the quantitative tube test (figures 3 and 4). The agglutinin titre usually rose and fell in a short time so a constant titre was hardly kept. The values of the titre agreed closely with the reactions of the rapid test (Table 4). That is, the positive reactors had titre higher than 1 : 40 and the negative reactors had titre lower than 1 : 20, except for a few cases in which some special individuals (nos. 142, 147, 154 and 127) reacting negatively had comparatively high titres, 1 : 40 to 1 : 160.

The cause of the frequent fluctuation is unknown. The phenomenon is just like the response to repeated injection of antigen. It is also known that the more the antigen is administrated, the more the antibody is produced in some extent (8). Therefore it may be caused by the various concentration of the causative organism derived from antagonist between the host and the parasite in the course of the infection. That is, the fluctuation of the agglutinin level may signify a continuous strife between them.

The fact that the number of the positive reactors by the rapid serum test decreased remarkablly in November and December agrees with that obtained from our previous experiment (5). In this period, many of the carriers presented lower titre of the agglutinin than in the other periods. It is not evident whether this specific decrease of the agglutinin is caused by a seasonal effect of winter or by fowl's physiological conditions connecting with the growth,

because both of the fowls which were employed for the present and previous experiments hatched in spring so that their ages were similar.

The phenomenon may have been due to some nutritional condition because the response to the stimulus of an antigen is affected by the ration of the animal. Axelrod (9)(10) mentioned the incomplete formation of antibodies in vitamin deficient states. Green (11) demonstrated that vitamin A deficiency depress the formation of the antibody. In our experiments, green vegetables in the diet were apt to be insufficient in winter.

The phenomenon may have been due to the physiological conditions related with the egg production, for the fowls began to lay from the beginning January of Circulating antibody concertration is affected by adrenocorticotropic hormone (12) (13), of which secretion from the pituitary gland is invited by gonad hormone (14). Furthermore the secretion may be affected by the day length as thatof the gonadtropic hormone is done (14).

The phenomenon may have been only due to the proper course of the infection. Anyway, it will be a little effective to point out the pullorum carrier by the agglutination in such season or age.

Though it has been presented (1) (3) (5) that there are three or four types of pullorum fowls, six types were obtained from the result of the repeated rapid serum agglutination during about one year from the baby age in this work (Table 1). These types conformed to the result of the tube test (Figs. 3 and 4). The fact that the rate of the positive reactor in the rapid test was hardly kept constant was chiefly due to the alternate change of the reactions in the fowls belonging to Types II, III and IV. As in Type III, the fact that the fowls reacted again positively after continuation of the negative reaction will mean latent infection lacking any clinical symptom even antibody formation during the time rather than repeated infection, which, however, is a question because they were fed in a pen. On Type V, similar presumption will be also made, for they were distinctly infected with the disease in the baby age.

On the relationship between both results from the agglutination and the isolation of Sal. pullorum.

From all of the fowls belonging to the first three types, *Sal. pullorum* was isolated. The carriers of Types I and II are easily discovered by the agglutination, for the positive reaction is shown very frequently. The fowls of Type III were all carriers, which may be hardly detected by the test because their positive reaction disappears for a long time.

On the contrary, from the second two types, the organism was isolated at a rate as low as 20 to 33 per cent (Table 2). The carriers of these types are more dificult to be detected by the agglutination because they react positively only a few times even in repeated tests. Therefore they will be more

important spreaders in fields. For the detection of the none-reacting carrier, the use of adrenalin seemed to have no effect (Table 6). In the other type, there was no carrier. For the isolation was conducted very carefully, it is thought that the fowls (44 per cent of the total) from which no organism was recovered had completely recovered from the disease.

The fowls reacting positively when sacrificed were all carriers but all of the negative reactors were not pullorum-free (Table 5). It must be considered in practical works that 22 per cent of the negative reactors, particularly two fowls (Nos. 135 and 139) in the type IV, were the carriers (Table 2).

Sexual difference of the attitudes to the pullorum infection.

Most of the females belonged to the first three types and most of the males were the other three types. Thirty-one per cent of the males and 81 per cent of the females were carriers (Table 2). Therefore the male seems to be more resistive to the disease than the female. This fact will involve something connecting with what the agglutinin levels of laying hens were apt to be higher than those of immature fowls (figures 3 and 4).

Conclusion and summary

The results obtained from this work will suggest the better use of the agglutination for the control of the pullorum disease in fields and will be summarized as follows.

1) Agglutinin was not produced as much as we could detect by the baby chicks until they reached ages of 25 days and 22 days in the natural and artificial cases, respectively. The less ability of the formation of the antibody may be one of the reasons why the severe infection occurs easily in the baby age.

2) In the natural infection, 38 per cent and 29 per cent of the total positive reactors began to show the reaction at ages of 25 and 32 days respectively, and most of them, 84 per cent, appeared before 53 days old. For these reasons it will be beneficial to the control of the pullorum disease that agglutination are carried out repeatedly for a month from 20 to 50 days of age.

3) After the positive reactors in the rapid serum tests reached the maximum level, 53 per cent of the total fowls, at 46 days of age, soon decreased till a level having some fluctuation. Sometimes they widely fluctuated to run away from the limits so that significant increase appeared at the ages of 79 days (in August), 302 days (in March) and 357 days (in April) and a significant decrease appeared from 193 to 221 days old (November to December).

4) Agglutinin titres measured by the tube method always fluctuated so they hardly kept constant levels. The attitudes of their fluctuations were

almost in conformity with those of the reactions by the rapid tests. The cause of the fluctuation occurring frequently is unkown. The positive reactor also remarkally decreased in November and December as in the case of our previous experiment, though it is not clear whether the phenomenon was due to the seasonal effects of winter.

5) The six types of the infected fowls were classified according to the beginning and the duration of the reaction to the rapid test. The characters of these types almost agreed with those of both results from the tube test and from the isolation of *Sal. pullorum.*

6) All of the fowls belonging to Types I, II and III and some of Types IV and V were pullorum carriers. Because those belonging to Types II, III, IV and V do not continue the positive reaction, repeated tests will be necessary for the detection of such carriers. Especially, the carriers of Types IV and V, which seem to be important spreaders of the bacteria, will be hardly detected even by the frequently repeated tests, for they react positively only a few times.

7) Females, as compared with males, were apt to keep higher titre and *Sal. pullorum* were more frequently isolated from them. They are more liable to become the carrier than the males.

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References

1) Imai, N. and Kamimura, T. : (1954). Sci. Report. Azabu Vet. Colleg, No. 1, 28–39.
2) Imai, N. et al. : (1955). Sci. Report. Azabu Vet. College, No. 2, 35–42.
3) Iwamori, H. et al. : (1952). Japan. J. Vet. Sci., **14**, 329.
4) Edwords, P. R. and Hull, F. F. : (1929). J. Am. Vet. Med. Assn., **26**, 705–768
5) Katsuno, M. and Fujita, J. : (1956). Tohoku J Agr Res., **7**, 31–34.
6) Suzuki, K. and Tanaka, N. : (1952). Japan. J. Vet. Sci., **14**, 329–330.
7) Mizuma, Y. (1956). Unpablished data.
8) Topley, W. W. C. and Wilson, G. S. : (1936). The Principles of Bacteriology and Immunity. 2nd Ed., 836–888, Edward Arnold and: Co., London
9) Axelrod, A.E. : (1952). Nutrition Rev., **10**, 353–357.
10) Carter, B. B. and Axelrod, A. E : (1948) Proc. Soc. Exp. Biol. Med., **67**, 416–417.
11) Green : (1933). Am. J. Hyg., **17**, 60.
12) Dougherty, White and Chase : (1944). Proc. Soc. Exp. Biol. Med., **56**, 28–29.
13) Chase, White and Dougherty : (1946). J. Immun., **52**, 101–112.
14) Takewaki, K. : (1944).Horumon. lst Ed., 54–130, Syunyodo, Tokyo.